

Survival and Outgrowth of *Clostridium sporogenes* Spores during Curing and Storage of Corned Beef with Reduced Levels of Sodium Chloride

ABSTRACT

Brines for curing meats contain salt and nitrite levels (up to 21% and 900 ppm, respectively) that inhibit outgrowth of spores. Brines containing lowered levels of salt were examined for their effect on *Clostridium sporogenes* spore outgrowth in cured corned beef during curing and subsequent storage (20°C). Outgrowth of *C. sporogenes* spores was monitored in the corned beef and also in Botulism Assay Medium (BAM) under various levels of sodium chloride and nitrite and different pH values. NaCl concentrations of 2.5% or less in cured corned beef had little effect on initial rates of growth, but 3.5% slowed the growth rate. Nitrite levels decreased during curing so that spores in corned beef containing 2.5% salt made with and without nitrite had similar rates of outgrowth during subsequent storage.

Decreasing the amount of sodium chloride in meat products would lessen dietary sodium intake and would contribute to reducing the incidence of hypertension and cardiovascular disease in susceptible individuals. However, use of sodium chloride can extend shelf life and prevent growth of certain pathogenic microorganisms.

Factors affecting growth and toxin production by *Clostridium botulinum* have been reviewed by Hauschild et al. (8), Holley (10), and Pierson et al. (13). Complete inhibition of growth of proteolytic strains requires a water activity equivalent to that produced by salt concentrations over 8.5%, a level much greater than that present in meat products (12). However, the curing brines contain inhibitory concentrations of salt and nitrite. The inhibition of clostridia by salt, nitrite, and pH was studied in media (11,15), pork slurries (7,14,16), and cured pork (12). The influence of salt concentration on clostridial growth was studied in dry and semi-dry sausages (3), frankfurters-bologna (17,19), and cooked corned beef (18). Few studies have been reported on non-fermented, cured meat products (8). Research from this laboratory showed that *Clostridium sporogenes* did not grow in frankfurters (19) but did grow after inoculation into cooked corned beef containing the same levels of salt (18). These data suggested the importance of the nitrite concentration in the spore environment although

the mechanism of nitrite inhibition and relationship of residual nitrite to growth remains unclear (2,7,13). The greater importance of temperature control compared to salt concentration has been shown for control of clostridia in meat products (14,16,18,19).

This paper follows the effect of salt concentration on the lag period and growth of *C. sporogenes* in an artificial medium at two pH values and with added sodium nitrite to better understand the interaction of these factors at low nitrite concentrations. The survival of *C. sporogenes* during a curing process closely resembling an industrial process with changing salt concentrations, and subsequent growth at 20°C is then followed in corned beef to determine whether curing brines which give different levels of salt in the cured product would affect survival and growth of *C. sporogenes*.

MATERIALS AND METHODS

Microbiological methods

C. sporogenes (NRRL # B1219) was used in these studies rather than *C. botulinum* because *C. sporogenes* is a non-toxic proteolytic organism closely related biochemically to *C. botulinum* (2). Spore suspensions were prepared in beef heart infusion (BHI) as described previously (18,19). Botulinum Assay Medium (BAM) (9) at 60% final volume was adjusted to a pH of 7.3 or 5.5 after autoclaving. Sodium nitrite (up to 250 ppm) and sodium chloride (up to 20%) solutions were sterilized by filtration, 1.0 ml of each was added to screw-capped tubes containing 3.0 ml of BAM medium, and sterile water was added, if necessary, to give 5.0 ml total. Spores were heat-shocked (80°C for 10 min) in 0.1% peptone-thioglycolate, and 1.0 ml was added to the tubes (10^3 spores/ml). Each treatment had a control and three inoculated tubes; each was replicated three times. Tubes were evacuated and flushed several times with a H₂:CO₂:N₂ (4:10:86%) gas mixture, then tightly sealed and incubated at 20°C for a maximum of 7 d. Spore outgrowth was monitored by measuring absorbance at 610 nm with a Bausch and Lomb Spectronic 20 Spectrophotometer. The lag period was determined by extrapolating the linear increase in absorbance back to the time when the absorbance was the same as the initial absorbance. The growth rate was estimated by the slope of the increasing absorbance with time during the log phase.

Corned beef

For each of three replications, beef bottom round was obtained from a local distributor and trimmed of visible fat and connective tissue. The meat was cut into 1.2-cm cubes, and 25 g portions were placed into vacuum pouches. Following vacuum sealing of the pouches, the meat was irradiated with 1 Mrad gamma radiation from a cesium 137 source (maximum temperature 2.7°C). After overnight storage at 6°C, the pouches were opened aseptically, and 0.3 ml of heat shocked spores (10^4 spores/g of meat) and 5.0 ml of filter-sterilized sodium chloride-sodium nitrite-sodium ascorbate solution were added. The pouches were vacuum-sealed (0.97 torr), meat was cured for 4 d at 6°C, and then stored at 20°C for up to 11 additional days.

During the storage period, pouches from each salt level were randomly selected for enumeration. Corresponding uninoculated control pouches were also sampled. To each pouch 60 ml of peptone-thioglycolate was added followed by a complete mixing in a Stomacher 400 for 1 min. Three ml were removed, diluted with peptone-thioglycolate, and plated with BAM agar. The plates were placed in Oxoid anaerobic jars which were flushed several times with the $H_2:CO_2:N_2$ gas mixture and incubated at 37°C.

Chemical analyses

Uninoculated pouches from each treatment were sampled on days 1, 4, and 6. Sodium content of the meat was obtained by dry ashing at 525°C, dissolving in nitric acid, and measuring by atomic absorption spectroscopy (1). Residual nitrite was estimated by the Griess reagent following the AOAC procedure (1) except that sulfanilic acid and 1-naphthylamine were added simultaneously.

RESULTS AND DISCUSSION

Growth in media with different salt and nitrite levels

Table 1 shows that decreases in pH from 7.3 to 5.5 increased the lag period over three-fold. Increases in salt levels delayed growth with the greatest change occurring between 2 and 3%. Growth varied greatly at pH 5.5 and 3-4% salt; one replicate showed no growth and was arbitrarily given the maximum time of 168 h. Rate of growth was greater at pH 7.3 and decreased sharply with increasing salt levels. *C. sporogenes* growth was similar to that reported for *C. botulinum* (11). Addition of 50 ppm or less of sodium nitrite greatly increased the lag time and decreased the growth rate relative to the no nitrite-no salt medium at either pH. Addition of 12.5, 25 or 37.5 ppm NO_2 to pH 5.5 BAM containing selected salt levels prevented growth during the time period studied (168 h) (data not shown). Addition of these NO_2 levels to pH 7.3 BAM resulted in very inconsistent occurrence of growth between replicate trials. However, the 12.5 and 37.5 ppm NO_2 with 1% salt BAM repeatedly exhibited delayed growth relative to the 1% salt-no nitrite BAM.

Survival in corned beef during curing

Pouches of meat were irradiated to destroy contaminating bacteria. The irradiated meat was held overnight before inoculation because preliminary trials indicated inhibitory effects from radiolytic products which would re-

TABLE 1. Lag period and growth rate of *C. sporogenes* in BAM medium when incubated at 20°C.

		Nitrite concentration (ppm)			
NaCl		0		50	
pH	(%)	Lag time (h)		Growth rate (Δ Abs/10 h)	
5.5	0	58 \pm 6	112 \pm 57	.047 \pm .034	.012 \pm .020
	1	60 \pm 7		.033 \pm .029	
	2	71 \pm 4		.036 \pm .022	
	2.5	89 \pm 17		.024 \pm .026	
	3	115 \pm 46		.011 \pm .011	
	4	117 \pm 45	168 ^a	.003 \pm .006	0 ^a
7.3	0	16 \pm 5	35 \pm 8	.38 \pm .12	.14 \pm .05
	1	16 \pm 4		.34 \pm .03	
	2	19 \pm 4		.18 \pm .07	
	2.5	23 \pm 2		.19 \pm .08	
	3	26 \pm 2		.12 \pm .02	
	4	28 \pm 2	132 \pm 62 ^b	.09 \pm .01	.01 \pm .01 ^b

Means ± standard deviations n=3.

^aNo growth after 168 h.

^bNo growth in 2 of 3 replicates after 168 h.

tard spore germination/outgrowth. This effect has been observed with non-spore forming bacteria (4).

The concentrations of NaCl and $NaNO_2$ during the curing process are shown in Fig. 1 (insets). The curing period was from day 0 to 4 at 6°C, storage was from day 4 to 15 at 20°C. Brines (20% w/w of meat) contained 900 ppm $NaNO_2$, 3000 ppm sodium ascorbate, and from 0 to 21% NaCl; the latter concentration equilibrated to produce corned beef with 3.5% salt. The 1.5% salt corned beef had an initial brine concentration of 9% which is at the inhibitory level for germination and outgrowth by spores of proteolytic clostridia (8,13). Initial 900 ppm sodium nitrite concentrations of the brine were also too high for growth (13). One sample was made without nitrite. Storage was chosen to be at 20°C to permit outgrowth (10).

Salt equilibrium in the meat essentially occurred in one day, with final concentrations close to those intended. The size of meat pieces used would simulate rate of cure

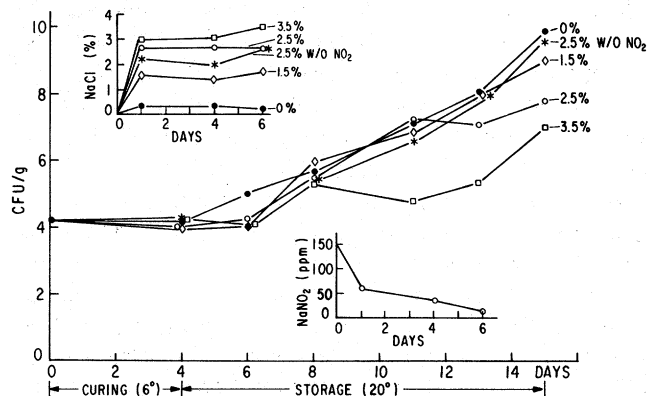


Figure 1. Growth of *C. sporogenes* spores during the curing and abuse storage (20°C) of corned beef. Upper inset shows the uptake of salt by the meat and lower inset shows the depletion of nitrite. Values are means of three trials.

distribution in a larger piece of meat that had been pumped or injected with brine (5). Residual nitrite declined rapidly, averaging 60 ppm nitrite after 1 d; nitrite depletion was slightly faster with higher salt concentrations (2). Ascorbate depletes nitrite rapidly (2,13). Large variations were observed between batches of meat, with one batch having less than 12 ppm detectable nitrite after 1 d. The average residual nitrite concentration was 13 ppm after 6 d.

When pouches of the cured meat were palced in 20°C storage on day 4, the salt-nitrite concentrations and temperature were favorable for spore outgrowth. Platings from day 4 indicated that counts were equivalent to the inoculation for all treatments (Fig. 1). The high salt and nitrite concentrations that contacted the spores had no lethal or lasting inhibitory effect.

After the second day of storage, spores in the 0% salt corned beef germinated and grew (day 6) while the spores in corned beef with higher salt levels had not grown. On the next sampling (day 8), all treatments had the same counts. With further storage, the 3.5% salt corned beef inhibited growth more then did the lower salt levels. During the three log cycles of growth over the first 9 d of storage, no inhibition was evident with 2.5% or less salt although at the final sampling an inhibitory effect was observed with the 2.5% salt concentraton. Growth of *C. sporogenes* in corned beef made without nitrite and with 2.5% salt was initially the same as in corned beef made with nitrite.

Relative decreases in rates of growth with increasing salt concentrations were much greater in BAM than in the meat. The data from the BAM medium would suggest that beef with its pH at 5.5-6.0 and low nitrite levels should not support growth. However, Fox (6) discussed the complex reactions of nitrite in meat, raising the possibility that spore inhibition by low levels of nitrite may not be the same in a medium and meat. At the same salt concentration and pH, BAM medium with 12.5 ppm added nitrite may have more effective inhibition than the corned beef with 13 ppm residual nitrite measured by the Griess reagent.

Spores were able to survive the high salt and nitrite in the curing brine and grew after the salt equilibration and nitrite depletion of curing. The growth in reduced salt and no-nitrite samples were similar to the normal 2.5% salt-nitrite samples, indicating that little inhibitory effect existed after curing when stored at a 20°C abuse temperature.

ACKNOWLEDGMENT

The authors acknowledge advice and assistance by members of the USDA Microbial Food Safety Research Group, ERRC, Philadelphia,

PA. Reference to a brand or firm name does not constitute endorsement by the U.S. Dept. of Agriculture over others of a similar nature not mentioned.

REFERENCES

1. AOAC. 1980. Official methods of analysis, 13th ed. Association of Official Analytical Chemists, Washington, DC.
2. Benedict, R. C. 1980. Biochemical bases for nitrite inhibition of *Clostridium botulinum* in cured meat. J. Food Prot. 43:877-891.
3. Collins-Thompson, D. L., B. Krusky, W. R. Usborne, and A. H. W. Hauschild. 1984. The effect of nitrite on the growth of pathogens during manufacture of dry and semi-dry sausage. Can. Inst. Food Sci. Technol. J. 17:102-106.
4. Dickson, J. S., and R. B. Maxcy. 1984. Effect of radiolytic products on bacteria in the food system. J. Food Sci. 49:577-580.
5. Fox, J. B., Jr. 1980. Diffusion of chloride, nitrite, and nitrate in beef and pork. J. Food Sci. 45:1740-1744.
6. Fox, J. B., Jr. 1984. Nitrite. Proceedings Meat Industry Research Conference. American Meat Inst. pp. 38-47.
7. Gibson, A. M., T. A. Roberts, and A. Robinson. 1984. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized cured meats. VI. Nitrite monitoring during storage of pasteurized pork slurries. J. Food Technol. 19:29-44.
8. Hauschild, A. H. W. 1982. Assessment of botulism hazards from cured meat products. Food Technol. 36:95-104.
9. Huhtanen, C. N. 1975. Some observations on a Perigo-type inhibition of *Clostridium botulinum* in a simplified medium. J. Milk Food Technol. 38:762-763.
10. Holley, R. A. 1981. Review of the potential hazard from botulism in cured meats. Can. Inst. Food Sci. Technol. J. 14:183-195.
11. Montville, T. J. 1983. Interaction of pH and NaCl on culture density of *Clostridium botulinum* 62A. Appl. Environ. Microbiol. 46:961-963.
12. Nordin, H. R., T. Burke, G. Webb, L. J. Rubin, and D. van Binnendyk. 1975. Effect of pH, salt and nitrite in heat processed meat on destruction and out-growth of P.A. 3679. Can. Inst. Food Sci. Technol. J. 8:58-66.
13. Pierson, M. D., and L. A. Smoot. 1982. Nitrite, nitrite alternatives, and the control of *Clostridium botulinum* in cured meats. CRC Crit. Rev. Food Sci. Nutr. 17:141-187.
14. Roberts, T. A., A. M. Gibson, and A. Robinson. 1981. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats. J. Food Technol. 16:239-266.
15. Roberts, T. A., and M. Ingram. 1973. Inhibition of growth of *Cl. botulinum* at different pH values by sodium chloride and sodium nitrite. J. Food Technol. 8:467-475.
16. Robinson, A., A. M. Gibson, and T. A. Roberts. 1982. Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats. V. Prediction of toxin production: Non-linear effects of storage temperature and salt concentration. J. Food Technol. 17:727-744.
17. Sofos, J. N. 1985. Influence of sodium tripolyphosphate on the binding and antimicrobial properties of reduced NaCl-communited meat products. J. Food Sci. 50:1379-1383,1391.
18. Whiting, R. C., R. C. Benedict, C. A. Kunsch, and D. Blalock. 1985. Growth of *Clostridium sporogenes* and *Staphylococcus aureus* at different temperatures in cooked corned beef made with reduced levels of sodium chloride. J. Food Sci. 50:304-307.
19. Whiting, R. C., R. C. Benedict, C. A. Kunsch, and J. H. Woychik. 1984. Effect of sodium chloride levels in frankfurters on the growth of *Clostridium sporogenes* and *Staphylococcus aureus*. J. Food Sci. 49:351-355.